Refractoriness to the effect of endothelin-1 in porcine ciliary arteries

Konieczka, K ; Flammer, A J ; Neutzner, A ; Schoetzau, A ; Binggeli, T ; Flammer, J

Abstract: PURPOSE: Endothelin-1 (ET) is an important molecule in vascular physiology. After an acute stimulation with ET, vessels are to some extent temporarily refractory to further stimulation. However, few details are known about this phenomenon. The aim of our study was to verify the existence of refractoriness in ophthalmic ciliary arteries and, if present, to analyze its time course. METHODS: Twenty freshly isolated porcine ciliary arteries were placed in a myograph system to measure isometric forces. Each vessel was stimulated with 10(-7) M ET twice. The experiment was performed in 5 groups of vessels, which differed in the time interval between the initial and the second stimulation with ET. The intervals were 15 min, 30 min, 1 h, 2 h, and 4 h, respectively. RESULTS: The vasoconstrictive response to re-exposure to ET was time-dependently reduced. The response was lowest after 15 min (22% of baseline response), and then the sensitivity slowly recovered and was finally normal again after 4 h. CONCLUSIONS: Our experiment with isolated porcine ophthalmic ciliary arteries revealed a refractoriness phase to ET after an acute stimulation with ET. This refractoriness was transient and disappeared after 4 h. The lowest response was observed in the group of vessels re-exposed 15 min after the first stimulation.

DOI: https://doi.org/10.1089/jop.2012.0060

JOURNAL OF OCULAR PHARMACOLOGY AND THERAPEUTICS
Volume 29, Number 5, 2013

DOI: 10.1089/jop.2012.0060

Refractoriness to the Effect of Endothelin-1 in Porcine Ciliary Arteries

Katarzyna Konieczka,[1] Andreas J. Flammer,[2] Albert Neutzner,[1] Andreas Schoetzau,[1] Tatjana Binggeli,[1] and Josef Flammer[1]

Abstract

Purpose: Endothelin-1 (ET) is an important molecule in vascular physiology. After an acute stimulation with ET, vessels are to some extent temporarily refractory to further stimulation. However, few details are known about this phenomenon. The aim of our study was to verify the existence of refractoriness in ophthalmic ciliary arteries and, if present, to analyze its time course.
Methods: Twenty freshly isolated porcine ciliary arteries were placed in a myograph system to measure isometric forces. Each vessel was stimulated with 10^{-7} M ET twice. The experiment was performed in 5 groups of vessels, which differed in the time interval between the initial and the second stimulation with ET. The intervals were 15 min, 30 min, 1 h, 2 h, and 4 h, respectively.
Results: The vasoconstrictive response to re-exposure to ET was time-dependently reduced. The response was lowest after 15 min (22% of baseline response), and then the sensitivity slowly recovered and was finally normal again after 4 h.
Conclusions: Our experiment with isolated porcine ophthalmic ciliary arteries revealed a refractoriness phase to ET after an acute stimulation with ET. This refractoriness was transient and disappeared after 4 h. The lowest response was observed in the group of vessels re-exposed 15 min after the first stimulation.

Introduction

Endothelin, identified in 1988 by Yanagisawa et al.,[1] is one of the strongest known endogenous vasoconstrictors. The predominant isoform in the endothelin family is endothelin-1 (ET). Under physiological conditions, ET is mainly synthesized by vascular endothelial cells. However, in hypoxia or other pathological conditions, ET can also be produced by other cells.[2–4] In humans, the effects of ET are mediated by 2 types of ET receptors: the type-A receptor (ET_A) and the type-B receptor (ET_B). ET_A receptors are found on vascular smooth muscle cells, and their stimulation leads to marked and sustained vasoconstriction by increasing cytoplasmatic calcium. ET_B receptors can be found on both endothelial cells and smooth muscle cells. ET_B receptors on endothelial cells mediate the release of vasodilatory nitric oxide and/or prostacyclin; however, stimulation of ET_B receptors on smooth muscle cells provokes vasoconstriction.[5]

Together with other vasoactive molecules and the autonomic nervous system, ET regulates vascular tone. Several factors modulate the production of ET, including shear stress,[6] hypoxia-inducible factor-1-alpha,[7] angiotensin II,[8,9] adrenaline,[10] inflammatory cytokines[11,12] and others.

It is obvious that any malfunction in this system locally or systemically can have pathophysiologic implications, and a relative increase in ET can lead to vasoconstriction or even vasospasm. Indeed, ET raises blood pressure[13] and induces vascular and myocardial hypertrophy,[14,15] both contributors to increased cardiovascular morbidity and mortality.[16] ET has been shown to be involved in the pathogenesis of many diseases.[4]

In the eye, the regulation of blood flow is different in distinct vascular beds. Retinal circulation is mainly regulated by endothelium-derived vasoactive factors, while choroid circulation is predominantly regulated by the autonomic nerve system.[17] Optic nerve head circulation is, like that of the retina, regulated by endothelial cells, but it is also under the direct influence of circulating hormones (including ET). These circulating hormones diffuse from fenestrated capillaries of the choroid into the optic nerve head and reach the vascular smooth muscle cells directly, by-passing the blood-retinal barrier.[17,18] This is the reason why an increased level

[1]Department of Ophthalmology, University of Basel, Basel, Switzerland.
[2]Division of Cardiovascular Diseases, Department of Internal Medicine, Mayo Clinic and College of Medicine, Rochester, Minnesota.

of ET in the circulating blood (as can be observed in many different autoimmune diseases such as multiple sclerosis) has a major effect on optic nerve head circulation but no or only a minimal effect on retinal or brain circulation. An increased level of ET seems to be implicated in the pathogenesis of many ocular diseases, particularly retinal vein occlusion,[19,20] glaucoma,[21] diabetic retinopathy,[22] and giant cell arteritis.[23]

Although ET obviously plays an important role, many basic aspects of its function are still poorly understood, including the question of the dose-response relationship. An acute rise in ET concentration may have a completely different effect from a chronic increase.

The mode of action of ET is different from other vasoconstrictors like adrenaline or angiotensin II. Once ET binds to its receptor, it will barely dissociate again.[24] After binding, ET is rapidly internalized together with its receptor.[25] Such an internalization of the ET-ET receptor complex influences local ET concentration to some extent and has a major impact on receptor density and thus ET sensitivity.

Although tachyphylaxis with ET was already observed in rat aortic rings in 1993,[26] this phenomenon was not taken into account in later *in vitro* and *ex vivo* study designs. On the contrary, in many studies investigating the effect of ET and its blockers on vascular function, vessels were exposed to stepwise incremental concentrations of ET. If the vasculature is refractory to ET, the results of such studies should be interpreted with caution, especially when high concentrations of ET are studied. Information about refractoriness is of relevance for physiology, pathophysiology, and pharmacology, especially in light of the fact that ET and its receptors are promising targets for future drugs.

The purpose of the present study was a) to test whether refractoriness indeed exists in ocular vessels and b) to estimate its time course. By working on isolated vessels, the effect of ET is separated from the influence of other regulatory systems, like the autonomic nerve system or neurovascular coupling.

Methods

Preparation of vessels

In adherence with the Association for Research in Vision and Ophthalmology (ARVO) Statement for Use of Animals in Ophthalmic and Vision Research, porcine eyes with surrounding tissue were obtained from a local slaughterhouse immediately after death and transported in cold modified Krebs-Ringer solution (NaCl 118 mMol, KCl 4.7 mMol, KH_2PO_4 1.2 mMol, $MgSO_4$ 1.2 mMol, $NaHCO_3$ 25 mMol, EDTA 0.026 mMol, $CaCl_2$ 2.5 mMol, Glucose 11.1 mMol).

Ciliary arteries were dissected under a microscope (Wild M3C; Wild Heerbrugg AG) and cut into 2-mm segments. Arterial rings were mounted in specially designed organ chambers for small vessels (myograph system[27]). In an organ chamber, two 40-μm tungsten wires were passed through the vessel's lumen and attached to a force transducer for isometric force measurements (Multi Wire Myograph System 610M; Danish Myo Technology). Mounted vessels were immersed in the myographs' organ chambers filled with Krebs-Ringer bicarbonate solution (37°C; 95% O_2, 5% CO_2) and stretched in a stepwise manner until the optimum passive tension was reached. The optimum passive tension was defined as the level of vascular wall tension at which contractions to 100 mM potassium chloride (KCl) became maximal. The vessels were then washed out with modified Krebs-Ringer solution before the following experimental protocols were conducted.

Experimental protocols

Each of 20 ciliary arteries was stimulated with ET twice. Quiescent vessels were first exposed to 10^{-7} M ET, then washed out with modified Krebs-Ringer solution, and then exposed to 10^{-7} M ET again. Five series of experiments were performed, which differed in the time interval between the initial and the second stimulation with ET: 15 min, 30 min, 1 h, 2 h, and 4 h, respectively. At the end of each experiment, vessels were exposed to 100 mM KCl to establish that they were still responsive to vasoconstrictor agents. In each series of experiments, 4 porcine ciliary arteries of 4 animals were used (one vessel per experiment).

Drugs and statistical analyses

ET was purchased from Sigma-Aldrich Chemie GmbH. It was dissolved in distilled water. Concentrations are expressed as final molar concentrations in the organ chambers.

Results are given as ratio ET_{sec}/ET_{ini}, where ET_{sec} represents the response to the second stimulation with ET and ET_{ini} represents the response to the initial stimulation with ET. To explore the ratio ET_{sec}/ET_{ini}, descriptive statistics including mean and standard deviation were calculated. A graphical description using boxplots was assessed. To compare ET_{sec}/ET_{ini} across all time points, a linear regression model was performed. All ratios were compared to 1. Results are also expressed as differences of means to 1 for each time point. Standard errors (SEs) and corresponding P values are also reported. P values and SEs were adjusted for multiple comparisons using the method of Dunnett.[28] A P value < 0.05 was considered to be statistically significant. All analyses were done using the statistical package R version 12.2.[29]

Results

The vasoconstrictive response to re-exposure to ET was time-dependently reduced in comparison to the initial ET stimulation (Fig. 1, Table 1). The vasoconstrictive response to the first exposure to ET was arbitrarily defined as 1 U. 15 min, 30 min, 1 h, and 2 h after initial stimulation, the vasoconstrictive response to the second stimulation with ET was significantly lower than 1. The response was lowest at the 15 min time point with a mean response of 0.22 (ie, 22% of baseline response) and then slowly recovered to 0.29, 0.63, and 0.80 for the 30-min, 1-h, and 2-h time points, respectively. At the 4-h time point, the response reached an average of 0.9, a value that was no longer significantly different from the baseline value of 1.

At the end of the experiments, all vessels were still completely responsive to 100 mM KCl.

Discussion

The present study demonstrateted that (1) after an acute stimulation with ET, vascular smooth muscle cells in the porcine ciliary arteries were temporarily partly refractory to further stimulation and (2) this refractory effect was

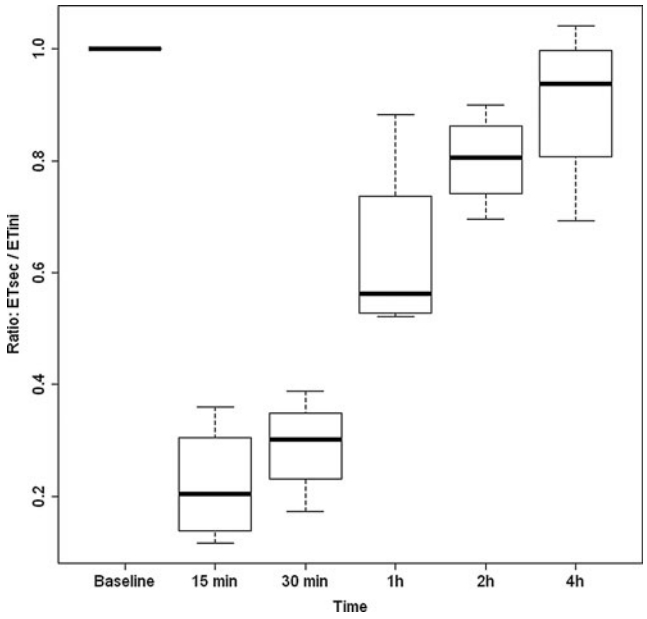

FIG. 1. Twenty isolated ciliary arteries were stimulated with 10^{-7} M endothelin-1 (ET) twice, on baseline condition and after a latency shown on the x-axis. The response to the initial ET stimulation (baseline) was arbitrarily defined as 1. The response to the second ET stimulation was expressed as the ratio ET_{sec}/ET_{ini} and shown on the y-axis. ($N=4$ vessels for each time point). Results are presented as box plots. ET_{ini} = vasoconstrictive response to the initial stimulation with ET-1 (baseline value); ET_{sec} = vasoconstrictive response to the second stimulation with ET-1 (re-exposure); Time = time interval between the initial and the second stimulation with ET-1.

strongest after 15 min and then attenuated and finally disappeared after 4 h.

After ET was discovered, Hirata et al. reported that the ET-induced increase in cytosolic free Ca^{2+} levels is reversibly absent or attenuated in ET-pretreated cultured cells.[30] Furthermore, a decreased vasoconstrictive effect after repeated ET administration was observed in rat aortic rings[26]; however, in this study, the time course of this phenomenon was not investigated. Desensitization to the effect of ET was also observed in rat mesenteric arterial smooth muscle cells,[31] in isolated guinea-pig ileum,[32] and in human small bronchi.[33] Concerning *in vivo* studies, however, the literature on the refractory phenomenon of ET is ambiguous. In anesthetized rats, the rapid development of desensitization to the hypotensive effects of ET was observed when repeated intravenous bolus injections of this peptide were given.[34] However, in the same study, the vasoconstrictive effects of ET did not undergo a fading phenomenon upon successive administrations of ET in pithed rats. This discrepancy between *ex vivo* and *in vivo* studies may be due to the different doses and therefore concentrations applied. But it is also possible that vessels denervated and isolated from their surroundings behave differently.

Table 1. Vasoconstrictive Response to the Second Stimulation with ET-1 in Relation to Baseline (ET_{INI}, Defined as 1.0) When Porcine Ciliary Arteries Were Re-exposed to ET-1

Time interval between baseline and re-exposure	*Ratio ET_{sec}/ET_{ini}*		
	Mean response	*SD*	P *value*
15 min	0.22	0.11	$P<0.001$
30 min	0.29	0.09	$P<0.001$
1 h	0.63	0.17	$P<0.001$
2 h	0.80	0.08	$P<0.007$
4 h	0.90	0.15	$P=0.13$

Results are presented as ratio ET_{sec}/ET_{ini} (mean, SD, and *P* value) for each time point.

(ET_{ini} = vasoconstrictive response to the initial stimulation with ET-1; ET_{sec} = vasoconstrictive response to the second stimulation with ET-1)

ET, endothelin-1; SD, standard deviation.

The observation that repeated stimulation of receptors by their agonists can lead to transient refractoriness is not specific for ET. Such phenomena are particularly observed when receptors are stimulated with high doses and may protect cells from overstimulation and potential damage. The exact mechanism of refractoriness to ET is not known. One important component might be the internalization of the ET-ET-receptor complex. These complexes are rapidly internalized by receptor-mediated endocytosis. After internalization, ET_A and ET_B receptors are targeted to different intracellular fates. Whereas the ET_A receptor follows the recycling pathway through the pericentriolar recycling compartment and then reappears at the plasma membrane,[35,36] the ET_B receptor is directed to lysosomes for degradation.[35] Therefore, a longer lasting signaling response can be achieved upon ET_A receptor stimulation.[35] The cytoplasmic carboxyl-terminal tail of the ET_A receptor is responsible for targeting this peptide to the recycling pathway.[35] This motif is lacking in the carboxyl-terminal region of the ET_B receptor, facilitating a divergent endocytic sorting of the ET_A receptor and ET_B receptor.[37] The time of internalization until reappearance of the receptors may correspond to the refractoriness.

However, receptor recycling may not be the only regulatory mechanism accountable for the refractory phenomenon of ET. ET receptors belong to the G protein-coupled receptors (GPCRs) family.[38] GPCR signaling receives negative feedback by G protein-coupled receptor kinases (GRK). ET signaling in arterial smooth muscle is tightly regulated by GRK2.[31] The inhibition of GRK2 reduces the extent of ET_A receptor desensitization, thus indicating that GRK2 may play an important role in the ET refractory phenomenon.

In addition, we cannot exclude that a certain component of the refractoriness is immanent to the experimental set-up. The vasoconstrictive response to the second ET stimulation might be reduced due to the fact that the resting tension at the time point of the second ET stimulation has not returned to the baseline levels yet. This, however, cannot explain the entire refractoriness observed in our study for the following reason: although the resting tension was still increased at the moment of the second ET stimulation, the total response (active response + resting tension) after the second ET stimulation was less than after the initial ET stimulation.

The observed refractoriness is most likely multifactorial. The purpose of the present study was to test the existence of refractoriness in an experimental set-up commonly used, particularly in drug studies. In such *ex vivo* experiments using a myograph system, vessels are often exposed to stepwise incremental concentrations of ET. Further studies are needed to explain the exact mechanism of this temporary

desensitization of vascular smooth muscle cells to ET and to distinguish between the roles of the ET_A and ET_B receptors.

What about the clinical implication of these findings? The level of ET in the circulating blood is increased in many diseases, including ocular diseases such as retinal vein occlusion,[19,20] diabetic retinopathy,[22] giant cell arteritis,[23] and retinitis pigmentosa.[39] Less is known about the extravascular ET concentration. It is increased, for example, in the aqueous humor of glaucoma patients[40] and is present in epiretinal membranes.[41] Even less is known about the time course of ET concentrations in tissues such as retina. However, acute hypoxia—as it occurs in many clinical conditions—stimulates ET expression locally, and without refractoriness, it can lead to a vicious circle.

Furthermore, information about refractoriness to ET is for sure of relevance for pharmacological vascular research. ET and its receptors are promising targets for future drugs.

What is the relationship between the concentration of ET used in our study and biological concentrations? The majority of ET produced in the endothelial cells is secreted abluminally, and only a small part is released into the lumen of the vessels. The resulting ET concentration in the circulating blood is in the range of pg/mL (1 pg/mL ET-1 = 0.4×10^{-12} M), whereas in the vascular wall, it is probably in the range of ng/mL[42] (1 ng = 0.4×10^{-9} M). However, little is known about the actual concentration in the extracellular space, between endothelial cells and smooth muscle cells. Even less is known about the corresponding concentration in the tissues under pathological conditions. Indeed, the concentration of ET used in our study may be higher than physiological concentration; however, it might be in the range reached under pathological conditions. We have chosen an ET concentration known to be within the range (upper half) of the concentration-response curve to ET in *ex-vivo* studies using a myograph system.[43,44]

In conclusion, we definitively proved the existence of refractoriness to ET in ocular vessels, at least in *ex-vivo* experiments and, for the first time, established an approximate time course of this interesting phenomenon. While the biological relevance of this finding needs to be established, it should already be taken into account in studies involving repeated ET applications.

Author Disclosure Statement

The authors report no conflicts of interest. No competing financial interests exist.

References

1. Yanagisawa, M., Kurihara, H., Kimura, S., et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature.* 332:411–415, 1988.
2. Desai, D., He, S., Yorio, T., Krishnamoorthy, R.R., and Prasanna, G. Hypoxia augments TNF-alpha-mediated endothelin-1 release and cell proliferation in human optic nerve head astrocytes. *Biochem. Biophys. Res. Commun.* 318: 642–648, 2004.
3. Yoshida, H., Imafuku, Y., Ohhara, M., et al. Endothelin-1 production by human synoviocytes. *Ann. Clin. Biochem.* 35: 290–294, 1998.
4. Khimji, A.K., and Rockey, D.C. Endothelin—biology and disease. *Cell Signal.* 22:1615–1615, 2010.
5. Remuzzi, G., Perico, N., and Benigni, A. New therapeutics that antagonize endothelin: promises and frustrations. *Nat. Rev. Drug Discov.* 1:986–1001, 2002.
6. Ishibazawa, A., Nagaoka, T., Takahashi, T., et al. Effects of shear stress on the gene expressions of endothelial nitric oxide synthase, endothelin-1, and thrombomodulin in human retinal microvascular endothelial cells. *Invest. Ophthalmol. Vis. Sci*. 52:8496–8504, 2011.
7. Powell, F.L., Kim, B.C., Johnson, S.R., and Fu, Z. Oxygen sensing in the brain—invited article. *Adv. Exp. Med. Biol*. 648: 369–376, 2009.
8. Emori, T., Hirata, Y., Ohta, K., Shichiri, M., and Marumo, F. Secretory mechanism of immunoreactive endothelin in cultured bovine endothelial cells. *Biochem. Biophys. Res. Commun.* 160:93–100, 1989.
9. Oriji, G.K. Angiotensin II-induced ET and PGI2 release in rat aortic endothelial cells is mediated by PKC. *Prostaglandins Leukot. Essent. Fatty Acids*. 61:113–117, 1999.
10. Nickel, T., Deutschmann, A., Hanssen, H., Summo, C., and Wilbert-Lampen, U. Modification of endothelial biology by acute and chronic stress hormones. *Microvasc. Res.* 78:364–369, 2009.
11. Yoshizumi, M., Kurihara, H., Morita, T., et al. Interleukin 1 increases the production of endothelin-1 by cultured endothelial cells. *Biochem. Biophys. Res. Commun*. 166:324–329, 1990.
12. Boesen, E.I., Sasser, J.M., Saleh, M.A., et al. Interleukin-1beta, but not interleukin-6, enhances renal and systemic endothelin production *in vivo. Am. J. Physiol. Renal. Physiol*. 295:F446–F453, 2008.
13. Vierhapper, H., Wagner, O., Nowotny, P., and Waldhausl, W. Effect of endothelin-1 in man. *Circulation.* 81:1415–1418, 1990.
14. Ito, H., Hirata, Y., Hiroe, M., et al. ET-1 induces hypertrophy with enhanced expression of muscle specific genes in cultured neonatal rat cardiomyocytes. *Circ. Res.* 69:209–215, 1991.
15. Barton, M., d'Uscio, L.V., Shaw, S., et al. ET(A) receptor blockade prevents increased tissue endothelin-1, vascular hypertrophy, and endothelial dysfunction in salt-sensitive hypertension. *Hypertension.* 31:499–504, 1998.
16. O'Leary, D.H., Polak, J.F., Kronmal, R.A., et al. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. *N. Engl. J. Med.* 340:14–22, 1999.
17. Flammer, J., Orgül, S., Costa, V.P., et al. The impact of ocular blood flow in glaucoma. *Prog. Retin. Eye Res.* 21:359–393, 2002.
18. Sossi, N., and Anderson, D.R. Blockage of axonal transport in optic nerve induced by elevation of intraocular pressure. Effect of arterial hypertension induced by angiotensin I. *Arch. Ophthalmol*. 101:94–97, 1983.
19. Iannaccone, A., Letizia, C., Pazzaglia, S., et al. Plasma endothelin-1 concentrations in patients with retinal vein occlusions. *Br. J. Ophthalmol.* 82:498–503, 1998.
20. Fraenkl, S.A., Mozaffarieh, M., and Flammer, J. Retinal vein occlusions: the potential impact of a dysregulation of the retinal veins. *EPMA J.* 1:253–261, 2010.
21. Emre, M., Orgül, S., Haufschild, T., Shaw, S.G., and Flammer, J. Increased plasma endothelin-1 levels in patients with progressive open angle glaucoma. *Br. J. Ophthalmol.* 89:60–63, 2005.
22. Morise, T., Takeuchi, Y., Kawano, M., Koni, I., and Takeda, R. Increased plasma levels of immunoreactive endothelin and von Willebrand factor in NIDDM patients. *Diabetes Care.* 18:87–89, 1995.

23. Pache, M., Kaiser, H.J., Haufschild, T., Lübeck, P., and Flammer, J. Increased endothelin-1 plasma levels in giant cell arteritis: a report on four patients. *Am. J. Ophthalmol.* 133:160–162, 2002.
24. Hirata, Y., Yoshimi, H., Takata, S., et al. Cellular mechanism of action by a novel vasoconstrictor endothelin in cultured rat vascular smooth muscle cells. *Biochem. Biophys. Res. Commun*. 154:868–875, 1988.
25. Resink, T.J., Scott-Burden, T., Boulanger, C., Weber, E., and Bühler, F.R. Internalization of endothelin by cultured human vascular smooth muscle cells: Characterization and physiological significance. *Mol. Pharmacol.* 38:244–252, 1990.
26. Hollenberg, S.M., Shelhamer, J.H., and Cunnion, R.E. Tachyphylaxis to the vasopressor effects of endothelin in rat aortic rings. *Am. J. Physiol.* 264:H352– H356, 1993.
27. Mulvany, M.J., and Halpern, W. Mechanical properties of vascular smooth muscle cells *in situ. Nature.* 260:617–619, 1976.
28. Dunnett, C.W. New tables for multiple comparisons with a control. *Biometrics.* 20:482–491, 1964.
29. R Development Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2010.
30. Hirata, Y., Yoshimi, H., Takaichi, S., Yanagisawa, M., and Masaki, T. Binding and receptor down-regulation of a novel vasoconstrictor endothelin in cultured rat vascular smooth muscle cells. *FEBS Lett.* 239:13–17, 1988.
31. Morris, G.E., Nelson, C.P., Standen, N.B., Challiss, R.A., Willets, J.M. Endothelin signaling in arterial smooth muscle is tightly regulated by G protein-coupled receptor kinase 2. *Cardiovasc Res.*85:424–433, 2010.
32. Miasiro, N., De Castiglione, R., and Paiva, A.C. Role of positions 9 and 10 in the endothelin molecule for biological activity and discrimination of receptor subtypes. *Eur. J. Pharmacol.* 278:103–109, 1995.
33. Adner, M., Cardell, L.O., Sjöberg, T., Ottosson, A., and Edvinsson, L. Contractile endothelin-B (ETB) receptors in human small bronchi. *Eur. Respir. J.* 9:351–355, 1996.
34. Le Monnier de Gouville, A.C., Mondot, S., Lippton, H., Hyman, A., and Cavero, I. Hemodynamic and pharmacological evaluation of the vasodilator and vasoconstrictor effects of endothelin-1 in rats. *J. Pharmacol. Exp. Ther.* 252:300–311, 1990.
35. Paasche, J.D., Attramadal, T., Sandberg, C., Johansen, H.K., and Attramadal, H. Mechanisms of endothelin receptor subtype-specific targeting to distinct intracellular trafficking pathways. *J. Biol. Chem.* 276:34041–34050, 2001.
36. Wang, J., Chiou, W.J., Gagne, G.D., and Wu-Wong, J.R. Internalization of type-Aendothelin receptor. *J. Cardiovasc. Pharmacol.* 36:S61–S65, 2000.
37. Paasche, J.D., Attramadal, T., Kristiansen, K., et al. Subtype-specific sorting of the ETA endothelin receptor by a novel endocytic recycling signal for G protein-coupled receptors. *Mol. Pharmacol.* 67:1581–1590, 2005.
38. Arai, H., Hori, S., Aramori, I., Ohkubo, H., and Nakanishi, S. Cloning and expression of a cDNA encoding an endothelin receptor. *Nature.* 348:730–732, 1990.
39. Cellini, M., Santiago, L., Versura, P., and Caramazza, R. Plasma levels of endothelin-1 in retinitis pigmentosa. *Ophthalmologica.* 216:265–268, 2002.
40. Shoshani, Y.Z., Harris, A., Shoja, M.M., et al. Endothelin and its suspected role in the pathogenesis and possible treatment of glaucoma. *Curr. Eye Res.* 37:1–11, 2012.
41. Roldán-Pallarés, M., Rollín, R., Mediero, A., et al. Immunoreactive ET-1 in the vitreous humor and epiretinal membranes of patients with proliferative vitreoretinopathy. *Mol. Vis.* 11:461–471, 2005.
42. Masaki, T., Yanagisawa, M., and Goto, K. Physiology and pharmacology of endothelins. *Med. Res. Rev.* 12:391–421, 1992.
43. Buckley, C.H., Hadoke, P.W., and O'Brien, C.J. Role of the endothelium in modulating functional responses of isolated bovine anterior ciliary arteries to vasoconstrictor agonists. *Br. J. Ophthalmol.* 82:826–829, 1998.
44. Cao, L., Zhang, Y., Cao, Y.X., Edvinsson, L., and Xu, C.B. Cigarette smoke upregulates rat coronary artery endothelin receptors *in vivo. PLoS One*. 7:e33008, 2012.

Received: March 20, 2012
Accepted: November 14, 2012

Address correspondence to:
Dr. Josef Flammer
Department of Ophthalmology
University of Basel
Mittlere Strasse 91
CH-4031 Basel
Switzerland

E-mail: jflammer@uhbs.ch